

Species Differences in the Metabolism and Disposition of Inhaled 1,3-Butadiene and Isoprene

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Species differences in sensitivity to carcinogenic effects from inhaled 1,3-butadiene might stem, at least in part, from differences in uptake, metabolism, and distribution of 1,3-butadiene. To examine this possibility, rats, mice, and monkeys were exposed to stepped concentrations of ^{14}C -labeled 1,3-butadiene and the chemically related compound, isoprene. Respiratory data were collected during exposure and were used to determine fractional uptake. Rates and routes of excretion of retained radioactivity were also determined and blood levels of potentially toxic metabolites were measured. In some cases, the concentrations of hemoglobin adducts were determined. For rodents, the tissue distribution of metabolites was examined. Some results from these continuing studies to date are: *a*) mice achieve higher blood concentrations of reactive metabolites than do rats; *b*) blood levels of toxic metabolites are lower in monkeys than in rodents; *c*) uptake and retention of 1,3-butadiene is nonlinear in the range where long-term toxicity studies have been conducted; *d*) the efficiency of production of reactive metabolites decreases with increased inhaled concentrations of 1,3-butadiene; *e*) repeated exposure to 1,3-butadiene does not induce the metabolism of 1,3-butadiene in rodents; *f*) hemoglobin adducts of 1,3-butadiene are potential dosimeters of exposure; and *g*) rats inhaling isoprene produce reactive metabolites analogous to those produced during inhalation of 1,3-butadiene. The available data indicate that major differences in the biological fate of inhaled 1,3-butadiene occur among species, and these differences, at least in part, account for those in species sensitivity to the toxicity of inhaled 1,3-butadiene.

Introduction

1,3-Butadiene is a major monomer in the rubber and plastics industry, where there is potential worker exposure. Incidences of neoplasia are higher in this industry than in the general population. Ascertaining a causal relationship between 1,3-butadiene exposure and the increased neoplasia is complicated, however, because there is exposure to a wide variety of potentially harmful compounds in this industry, including several known human carcinogens. Thus, the potential contribution of 1,3-butadiene to the increased incidences cannot be separated easily from the contributions of other chemicals.

The threshold limit value (TLV) for occupational exposure to 1,3-butadiene had been set at 1000 ppm. Therefore, the results of an oncogenesis study in Sprague-Dawley rats (1) showing 1,3-butadiene to be a weak carcinogen at 8000 ppm over 2 years did not cause great concern. In that study, increased incidences of mammary tumors, thyroid follicular adenomas, uterine tumors, and exocrine pancreatic adenomas were noted.

The results of a more recent study sponsored by the National Toxicology Program (NTP) (2), however, caused more concern. There was marked increased incidence of primary tumors in B6C3F₁ mice in both sexes exposed to 625 and 1250 ppm for only 60 to 61 weeks. The study was stopped earlier than the originally planned 2 years because tumors in the exposed mice caused excessive mortality. These murine tumors included lymphomas, hemangiosarcomas, alveolar/bronchiolar adenomas and carcinomas, acinar cell carcinomas, granulosa cell tumors, forestomach tumors, and hepatocellular adenomas and carcinomas. Thus, there was a great difference in sensitivity to the carcinogenicity of inhaled

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1,3-butadiene between mice and rats and also major differences in the target tissues at risk.

The chemical disposition program of the NTP conducts both prospective and retrospective studies that will help design and interpret toxicity studies. The results of the two carcinogenicity studies with 1,3-butadiene raised the question of whether the observed difference in species sensitivity could be related, at least in part, to differences in the disposition of inhaled 1,3-butadiene between the two species. Could differences in the absorption, distribution, metabolism, or excretion of 1,3-butadiene play a role in the species susceptibility to 1,3-butadiene carcinogenesis? In order to answer this question, the disposition of inhaled 1,3-butadiene was investigated.

Initial experiments were designed *a)* to determine the uptake and retention of inhaled ^{14}C -butadiene and the retention of its metabolites by rats and mice over a range of exposure concentrations, *b)* to identify the routes and half-times for elimination of the absorbed 1,3-butadiene-derived radioactivity, and *c)* to identify 1,3-butadiene metabolites in the blood of both species. In addition, studies were conducted to determine the distribution of the 1,3-butadiene-derived radioactivity in tissues, as well as the rates of elimination of this material from the tissues. These initial comparative studies have been extended to include studies of the absorption, metabolism, and elimination of inhaled 1,3-butadiene from cynomolgus monkeys. The production of active metabolites, especially those that have been shown by others to be genotoxic, were compared among the three species.

In addition to the uptake, retention, and disposition studies, the potential of 1,3-butadiene metabolites to form adducts with hemoglobin has been investigated. Hemoglobin adducts have been proposed as markers of exposure. The correlation of hemoglobin adduct levels with 1,3-butadiene metabolites in the blood is being established.

Because repeated exposure to a chemical may alter the way that chemical is handled due to induction or inhibition of metabolic enzymes, rats and mice were exposed repeatedly to 1,3-butadiene. The metabolism of 1,3-butadiene was examined in tissues from the exposed animals and was compared to tissues from animals not previously treated.

1,3-Butadiene is one of several related compounds used in the rubber industry. Structurally related compounds include isoprene (2-methyl-butadiene) and chloroprene (2-chloro-butadiene). Isoprene, a monomeric unit of terpenes, has been detected as a normal constituent of human and rodent exhaled air (3,4). Since the acute toxicity of isoprene is similar to 1,3-butadiene, we have conducted toxicokinetic studies on the fate of inhaled isoprene in rats and compared the results of absorption, metabolism, and excretion to those obtained from studies of 1,3-butadiene. Metabolism by mice and monkeys is also being investigated, as well as the generation of hemoglobin adducts.

Results from the inhalation studies of 1,3-butadiene

by rodents have been published (5-7), as have those from studies on the inhalation of isoprene by rats (8). A major purpose of the present report is to compare the results obtained using rodents with those from continuing studies on the toxicokinetics of inhaled 1,3-butadiene and isoprene in monkeys.

Methods

1,3-[1- ^{14}C]Butadiene with a radiochemical purity of > 99% was used. Rodents were exposed by the nose-only mode. Respiratory data (breathing frequency and tidal volume) were obtained for selected rodents during exposure. The methods used for rodents have been described (5,8), and these references should be consulted for experimental details. Monkeys (*Macaca fascicularis*, 5-7 kg males) were also exposed by the nose-only mode. Experimental details were the same as for rodents except as noted below. The exposure system used (Fig. 1) was a modification of one described for rats (9) except that, because radiolabeled 1,3-butadiene was used, a gas chromatograph was not needed. Also, instead of a pump to draw air past the monkey's nose, the respiratory action of the monkey was used for this purpose. Monkeys were anesthetized with pentobarbital during exposure. Before exposure, monkeys were fitted with an arterial catheter that was used both to introduce the anesthetic during exposure and to withdraw blood samples. Respiratory data (breathing frequency and tidal volume) for the monkey were obtained during exposure as described previously (10). The exposure protocols for rodents and monkeys are summarized in Figure 2.

Results

The rate of uptake of butadiene at approximately 10 ppm in mice was substantially greater than for rats or monkeys when normalized to body weight (Table 1). A greater rate of uptake for mice compared to rats was observed for inhaled 1,3-butadiene concentrations rang-

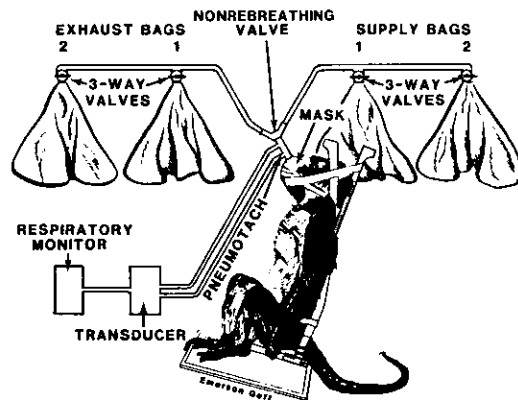


FIGURE 1. System for exposing monkeys by the nose-only route to vapors.

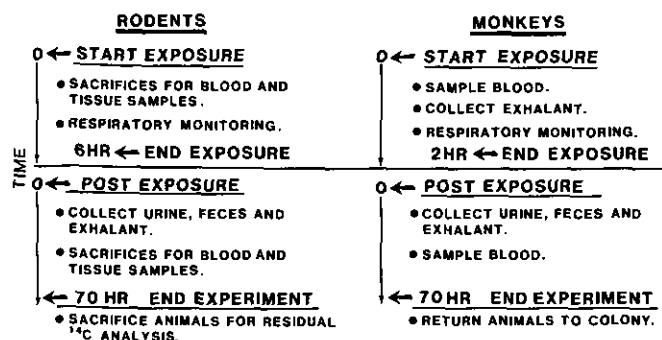


FIGURE 2. Summary of exposure protocols for toxicokinetics of inhaled 1,3-butadiene or isoprene in rodents and monkeys.

ing from 0.08 to 1000 ppm (Table 2). Data enabling the inclusion of monkeys in this comparison are being obtained in continuing exposures. Except for 1,3-butadiene at low inhaled concentrations (< 10 ppm) in mice, retention of butadiene- and isoprene-introduced ¹⁴C after a 6-hr exposure was not proportional to the concentration of inhalant (Fig. 3).

The route of elimination of 1,3-butadiene-introduced ¹⁴C after exposure of rodents was dependent to some extent on the exposure concentration, but elimination was always largely via the urine (Table 3). For monkeys, elimination was mostly by CO₂ exhalation after exposure to 10 ppm 1,3-butadiene. The toxicological significance of this observation is not clear; however, since the initial metabolism of 1,3-butadiene probably involves a mutagenic epoxide (11), whether or not CO₂ is the ultimate product (Fig. 4).

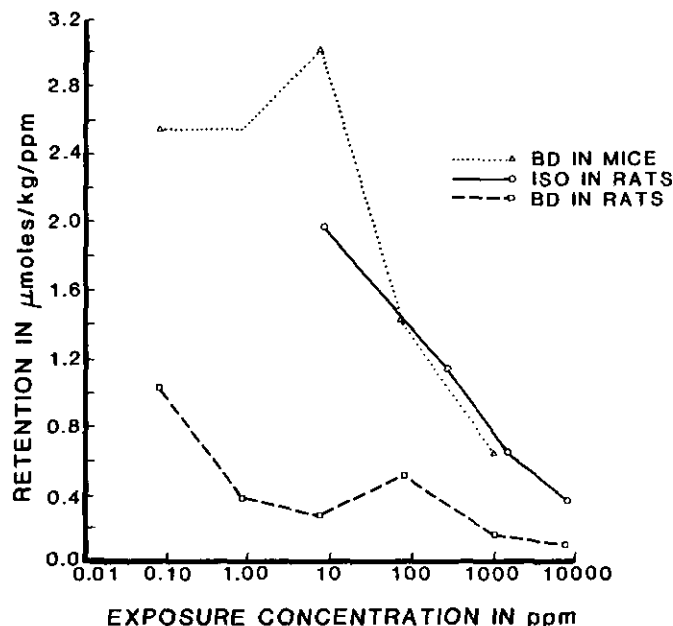


FIGURE 3. Efficiency of retention of 1,3-butadiene in rats and mice and of isoprene in rats after 6-hr exposure to concentration from 0.01 to 10,000 ppm. The ordinate has been normalized to inhaled concentration in ppm and thus stands for efficiency of uptake.

Total radioactivity in tissues after inhalation of ¹⁴C-labeled 1,3-butadiene at 700 ppm and 70 ppm by rats and mice, respectively, showed that mouse tissues contained 15 to 100 times higher concentrations of 1,3-butadiene-introduced ¹⁴C per μmole of inhaled 1,3-butadiene than did rat tissues. Major differences in storage depots

Table 1. Uptake of 1,3-butadiene inhaled at approximately 10 ppm by mice, rats, and monkeys.^a

Species	Exposure duration, hr	Animal mean mass, kg	1,3-Butadiene			
			Inhaled ppm	Inhaled μmole/hr/10 ppm	Retained, μmole/hr/10 ppm	Retained normalized to body mass, μmole/hr/10 ppm/kg
Mouse	6	0.028	7.8	0.70	0.09	3.30
Rat	6	0.400	7.8	4.40	0.19	0.46
Monkey	2	6.200	10.0	16.40	3.20	0.52

^aThe last three columns are normalized to 10 ppm and 1-hr exposure to facilitate direct comparison among exposures of different concentrations and exposure durations.

Table 2. Inhaled dose of 1,3-butadiene in rats and mice inhaling for 6 hr (mean ± SE).

Exposure concentration, ppm	Dose, μmole/kg ^a	
	Rats	Mice
0.08	0.08 ± 0.01*	0.2 ± 0.02
0.78	0.3 ± 0.01*	2 ± 0.2
7.2	2 ± 0.1*	22 ± 4
72	40 ± 3*	110 ± 11
1000	160 ± 10*	650 ± 50

^aDose is micromoles retained 6-hr postexposure.

*Significantly different ($p < 0.05$) from mice exposed to the same 1,3-butadiene concentration using Student's *t*-test.

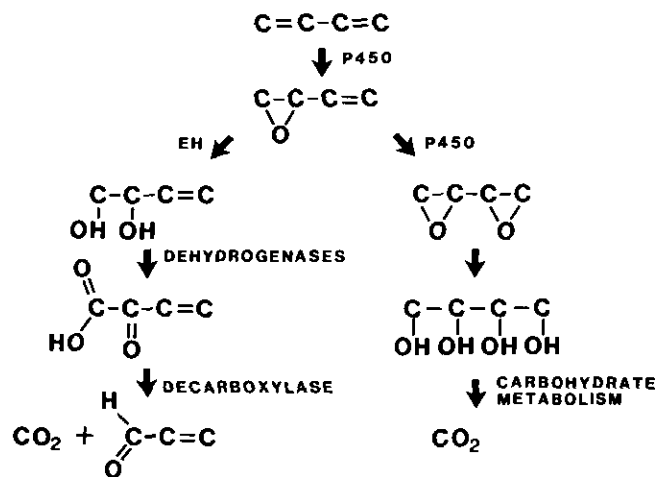
between the species or increased association of radioactivity with target tissues was not observed (6).

The levels of potentially genotoxic materials in the blood of mice inhaling approximately 10 ppm 1,3-butadiene was much higher than those in rats or monkeys after 2 hr of exposure (Table 4). These metabolites were tentatively identified, based on codistillation *in vacuo* with standards. HPLC analysis of these metabolites from monkey exhalant obtained during exposure to 10 ppm 1,3-butadiene showed coelution of > 10% with the genotoxic monoepoxide of 1,3-butadiene.

In experiments to determine if repeated exposure to

Table 3. Routes of elimination of metabolites of 1,3-butadiene after exposure (% of total metabolites eliminated in approximately 70-hr post-exposure) (mean \pm SE).

Species	1,3-Butadiene, ppm	Exposure duration, hr	Urine	CO ₂	Feces
Monkey	10	2	39 \pm 5	56 \pm 9	0.8 \pm 0.6
Mouse	7.2	6	58 \pm 15	32 \pm 3	10 \pm 2
	78	6	79 \pm 15	5 \pm 1	16 \pm 2
	1000	6	66 \pm 4	29 \pm 1	5 \pm 2
Rats	78	6	74 \pm 4	18 \pm 5	8 \pm 2
	1000	6	54 \pm 10	42 \pm 1	4 \pm 1

**FIGURE 4.** Some possible pathways for metabolism of 1,3-butadiene to carbon dioxide.

1,3-butadiene could induce 1,3-butadiene metabolism, rats and mice were exposed to 7600 or 750 ppm of butadiene, respectively, for 6 hr/day for 5 days (7). No induction of 1,3-butadiene metabolism was found in lung or liver. In fact, repeated exposures resulted in an approximate 50% depression of lung 1,3-butadiene metabolism in both rats and mice.

Rats and mice were injected IP with ¹⁴C-labeled 1,3-butadiene in corn oil to determine levels of hemoglobin adducts (12). The injected dose was distributed over 2 or 3 days so that the daily dose was 100 μ mole/kg body weight. This led to nearly linear increases in adduct levels with the injected dose. Single injected doses greater than 100 μ mole/kg body weight did not give linear responses, probably because of higher proportional elimination of 1,3-butadiene in the exhalant for larger injected doses (Fig. 5). Because the 1,3-butadiene for the method development work on the hemoglobin adducts was IP, comparisons to tissue distribution of 1,3-butadiene metabolites after inhalation are not warranted.

Isoprene (2-methyl-1,3-butadiene) undergoes metabolism to mutagenic products similar to those from 1,3-butadiene, namely, mono- and diepoxides (8). At roughly comparable inhaled concentrations, blood levels of mutagenic metabolites in rats after inhalation of isoprene were higher than after inhalation of 1,3-butadiene (Table 5).

Table 4. Blood epoxide levels of rats, mice, and monkeys after inhalation of 1,3-butadiene for 2 hr.

Species	Exposure 1,3-butadiene concentration, ppm	Normalized blood epoxide, pmole/mL/ppm
Mouse	7.8	77
Monkey	10	0.13
Mouse	78	26
Rat	78	5.2

Summary and Conclusion

Some important results are summarized as follows: *a)* Mice, which are more sensitive to the carcinogenic activity of 1,3-butadiene than are rats, achieve higher tissue levels of reactive metabolites than do rats exposed to the same exposure concentration. *b)* Blood levels of toxic metabolites (and probably hemoglobin adduct levels) appear to be lower in monkeys after inhaling 1,3-butadiene than they are in either rodent species. *c)* Uptake and retention of 1,3-butadiene is nonlinear in the range where long-term toxicity studies have been conducted. *d)* Although reactive metabolites are produced at all exposure concentrations, the efficiency of production of these metabolites appears to decrease with increased

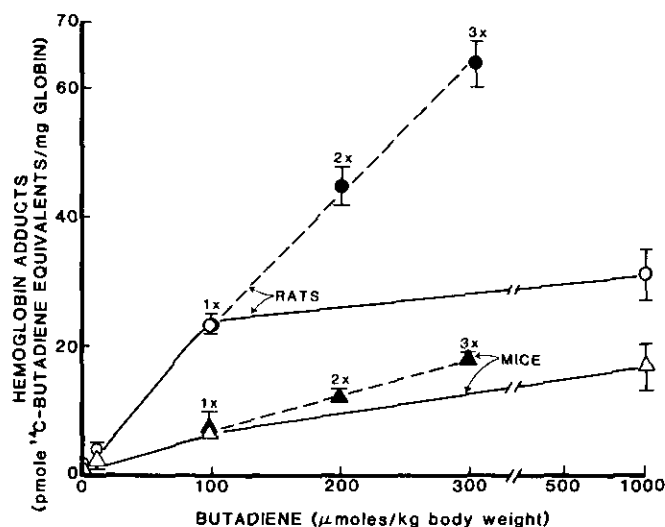
**FIGURE 5.** Levels of ¹⁴C-butadiene metabolite adducts formed with globin in mice (Δ) or rats (\circ) 24 hr after a single IP injection (1x, solid lines) of various amounts of ¹⁴C-butadiene dissolved in corn oil or two (2x) or three (3x) (dotted lines) daily injections of 100 μ mole ¹⁴C-butadiene/kg body weight into mice (Δ) or rats (\bullet). Data points are means \pm SE ($n = 3$).

Table 5. Blood concentrations of mutagens in rats after inhalation of isoprene or 1,3-butadiene, pmole/mL blood.^a

Vapor	Vapor concentration, ppm	Diepoxide ^a	Epoxide	Diepoxides/ppm	Epoxides/ppm
Isoprene	8	500	—	63	—
	260	10000	—	38	—
	1480	21000	—	14	—
	8200	23000	—	2.8	—
1,3-Butadiene	78	100	400	1.3	5.1
	1000	1000	4000	1	4

^aAssuming worst-case scenario wherein all radioactivity in blood that has volatility similar to the epoxide or diepoxide is that compound. Volatilities were determined using cryogenic vacuum distillation (13).

concentrations of inhaled 1,3-butadiene. *e*) Repeated exposures to 1,3-butadiene does not induce its metabolism in either rats or mice. *f*) Hemoglobin adducts have the potential to serve as dosimeters of exposure. Continuing research is directed toward developing sensitive chemical methods for detecting these adducts. *g*) Studies with isoprene have revealed that metabolites analogous to those produced from 1,3-butadiene are produced in rats during exposure to isoprene. This has led to the prediction that isoprene may have similar carcinogenic properties to those observed for 1,3-butadiene. This hypothesis is currently being tested in NTP-sponsored studies.

Data from four studies are in substantial agreement with those of Laib et al. (14) who used different methodologies to examine 1,3-butadiene toxicokinetics. Taken as a whole, the available data show important differences in the fate of inhaled 1,3-butadiene among different animal species. The direction of these differences indicate they may explain, at least in part, the differences in sensitivity among species to the toxic effects of inhaled 1,3-butadiene.

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